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From the above results it is seen that the melanophores in the skin of *Amiurus* react to direct stimulation by adrenalin. They are also subject to nervous control, and this control is mediated through the eye. There is also a suggestion of the secretion of a hormone under certain conditions and of its influence on the melanophores.

* Contributions from the Zoological Laboratory of the Museum of Comparative Zoölogy at Harvard College, No. 306.

FURTHER EXPERIMENTS ON THE SEX OF PARTHENOGENETIC FROGS

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It seemed necessary to furnish proof that by the methods of artificial parthenogenesis not only normal larvae can be produced from unfertilized eggs but that these larvae can also develop into full sized normal adults. This task is difficult to accomplish in sea urchins and thus far only Delage has reported that he has succeeded in raising one parthenogenetic larva of a sea urchin to the sexually mature form.

The possibility of producing artificial parthenogenesis in the eggs of the frog by the method of puncture, as demonstrated in the experiments of Guyer and of Bataillon, seemed more promising. The writer has made use of this method for deciding the question whether such frogs can reach the adult size, and determining their sex. He has now raised twenty leopard frogs to an age of from ten to eighteen months, and nine of these frogs are still alive. Some of these male frogs have reached the full size of the adult male leopard frog. *We are, therefore, entitled to say that the frogs produced by artificial parthenogenesis can develop into adults of full size and of an entirely normal character.*

Loeb and Bancroft¹ tried to ascertain the sex of a parthenogenetic frog immediately after metamorphosis but found the gonads in the intermediate stage, i.e., testes containing a few eggs, though it was obvious that the frog was developing into a male. It was clear that older frogs were needed for the decision of the problem of sex. The writer has been able to ascertain the sex in nine frogs of the age of from ten to eighteen months, and in all of these the ambiguity inherent in the younger frogs had disappeared. He has already reported that the first two of these parthenogenetic frogs has normal mature testes containing fully developed spermatozoa.² No eggs were found in these testes.

The next four frogs examined were also males, so that the problem seemed settled when a year ago last summer one parthenogenetic frog, sixteen months

old, was found whose gonads were macroscopically and microscopically well developed ovaries. The next frog was again a male. Although the possibility of an error in method seemed excluded the writer did not wish to publish the fact that both sexes appear in parthenogenetic frogs without having checked the result by a new series of experiments.

These experiments were started in February, 1917. The same precautions as in the older experiments were used. Copulating females which had not yet laid any eggs were separated from their males and kept separated for at least twenty-four hours. The females were repeatedly washed with water during the time of isolation, and directly before the experiment were submerged in 90% alcohol and left there to die. They were taken out, their abdominal cavity was opened with sterilized instruments and the oviduct laid bare. The eggs were taken out from the oviduct with sterilized instruments, and precautions were taken that the eggs did not come in contact with the hands of the experimenter or with the skin or outside of the frog. Alternate lots of about 50 to 100 eggs were punctured or kept untreated as controls. None of these non-treated eggs ever developed. From the punctured, unfertilized eggs ten developed into frogs, of which nine are still alive. The tenth was killed December 21 and the microscopic examination of its gonads showed that it was a female. This leaves then no doubt that both sexes can be produced from the unfertilized eggs of the frog. We have thus far obtained seven male frogs and two females, while the determination in two was missed by accident.

How can we account for the production of both sexes? The diploid number of chromosomes in the frog seems to be 26, according to Swingle,³ and, therefore, the haploid number 13. The question then arises: Do we find the diploid or haploid number of chromosomes in the cells of the parthenogenetic frog? Brachet⁴ found the diploid number in the somatic cells of a parthenogenetic tadpole eighteen days old, but, of course, it was out of the question to ascertain the sex of the tadpole.

The gap can be filled by counting the chromosomes in the fully developed parthenogenetic frogs. Thus far the sections of the testes of only one of the writer's parthenogenetic frogs have been examined cytologically. This male was seventeen months old, had reached the full size of the adult, and had large testes with ripe spermatozoa. Prof. R. Goldschmidt, who was good enough to examine some of the sections, counted over 20 chromosomes, and there can be no doubt that this parthenogenetic male frog possessed the diploid number of chromosomes. The writer has not yet been able to ascertain whether the nuclei of the female frogs have the haploid or diploid number.

It is not known whether the female or male is homozygous for sex in the frog. If the female were homozygous it would mean that the haploid number of chromosomes would be $12 + x$ and the diploid $24 + 2x$. In this case only a female could have the diploid number since $2x$ would determine a female. Since we find the diploid number of the male parthenogenetic

frog the assumption of homozygosity of the female is inadequate if not excluded. If we assume that the female is heterozygous for sex, and that it has the chromosome constitution $24 + x + y$ (where y may be missing), the male must have the chromosome constitution $24 + 2x$. The haploid number in the egg would be⁵ $12 + x$, and the diploid number either $24 + 2x$ or $24 + x + y$. The diploid number $24 + 2x$ would give rise to a male, while a female might be produced by either the haploid number $12 + x$ or the diploid number $24 + x + y$. It is, therefore, of some interest to find out whether or not the female has the haploid number $12 + x$ chromosomes. It is useless to enter into further speculation until this point is decided, which the writer hopes may be possible in the near future.

Summary.—The author has raised twenty leopard frogs produced by the methods of artificial parthenogenesis from unfertilized eggs to the age of from ten to eighteen months. Nine of these frogs are still alive. Some have reached the size of the full grown normal adult male. Both sexes are represented among the parthenogenetic frogs. Seven of the nine older frogs whose gonads were examined were males, and two were females. The parthenogenetic males possess the diploid number of chromosomes.

¹ Loeb, J., and Bancroft, F. W., *J. Exp. Zool., Wistar Inst., Philadelphia*, **14**, 1913, (275); **15**, 1913, (379).

² Loeb, J., these PROCEEDINGS, **2**, 1916, (313); *The Organism as a Whole*, New York, 1916.

³ Swingle, W. W., *Biol. Bull., Wood's Hole*, **33**, 1917, (70).

⁴ Brachet, A., *Arch. Biol., Paris-Bruxelles*, **26**, 1911, (362).

⁵ The other haploid number $12 + y$ may be left out of consideration for the present since it is possible that such eggs may not be able to develop.

THE RESOLVING POWERS OF X-RAY SPECTROMETERS AND THE TUNGSTEN X-RAY SPECTRUM

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This work was undertaken at the University of Iowa with the purpose of determining the wave lengths and the number of lines in the X-ray spectrum of tungsten with greater precision than had heretofore been done.

The method adopted was the well known photographic one in which the crystal is slowly rotated so that it will progressively pass through all the angular positions which are required for reflection of the incident X-rays as demanded by the formula $n\lambda = 2d \sin \theta$, in which n is the order of the spectrum, λ the wave length, d the grating constant, and θ the glancing angle of reflection.